PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference P1113PC00	FOR FURTHER ACTION	See item 4 below
International application No. PCT/EP2004/000856	International filing date (*day/month/year*) 30 January 2004 (30.01.2004)	Priority date (*day/month/year*) 30 January 2003 (30.01.2003)]
International Patent Classification (IPC) or national classification and IPC [7] C12Q 1/68, 1/68		
Applicant EPIGENOMICS AG		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☒	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 05 August 2005 (05.08.2005)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 740 14 35	Authorized officer Agnes Wittmann-Regis Telephone No. +41 22 338 89 70

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/000856	30.01.2004	30.01.2003

International Patent Classification (IPC) or both national classification and IPC
C12Q1/68

Applicant
EPIGENOMICS AG

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

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Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was field, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The following document has not been furnished:

   ☒ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2-10,13,15-20,28,29,31-36
	No:	Claims	1,11,12,14,21-27,30,37-40
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-40
Industrial applicability (IA)	Yes:	Claims	1-40
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: US-B-6 265 1711 (HERMAN JAMES G ET AL) 24 July 2001 (2001-07-24)

D2: WO 01/44504 A (FOX JAYNE CATHERINE ; HAQUE KEMAL (GB); LITTLE STEPHEN (GB); ASTRAZENE) 21 June 2001 (2001-06-21)

D3: WO 00/70090 A (UNIV SOUTHERN CALIFORNIA) 23 November 2000 (2000-11-23)

D4: OLEK ALEXANDER ET AL: "A modified and improved method for bisulphite based cytosine methylation analysis" NUCLEIC ACIDS RESEARCH, vol. 24, no. 24, 1996, pages 5064-5066, XP002106408 ISSN: 0305-1048

D5: WORM JESPER ET AL: "In-tube DNA methylation profiling by fluorescence melting curve analysis" CLINICAL CHEMISTRY, vol. 47, no. 7, July 2001 (2001-07), pages 1183-1189, XP002298308 ISSN: 0009-9147

D6: WO 99/60007 A (HAMILTON ALAN LEWIS ; SHCHEPINOV MIKHAIL SERGEEVICH (GB); SOUTHERN EDW) 25 November 1999 (1999-11-25)

D7: WO 97/37041 A (SEQUENOM INC) 9 October 1997 (1997-10-09)

D8: OLEJNIK J ET AL: "Photocleavable peptide-DNA conjugates: synthesis and applications to DNA analysis using MALDI-MS" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 27, no. 23, 1 December 1999 (1999-12-01), pages 4626-4631, XP002220753 ISSN: 0305-1048

D9: WO 01/76451 A (PIEPENBROCK CHRISTIAN ; BERLIN KURT (DE); EPIGENOMICS AG (DE); OLEK AL) 18 October 2001 (2001-10-18)

D10: YU ET AL: "SPECIFIC INHIBITION OF PCR BY NON-EXTENDABLE OLIGONUCLEOTIDES USING A 5' TO 3' EXONUCLEASE-DEFICIENT DNA POLYMERASE" BIOTECHNIQUES, EATON PUBLISHING, NATICK, US, vol. 23, no. 4, October 1997 (1997-10), pages 714,716-720, XP001041499 ISSN: 0736-6205

D11: US-A-5 866 336 (NAZARENKO IRINA A ET AL) 2 February 1999 (1999-02-02)

D12: WO 00/46398 A (KAY PETER H ; UNIV WESTERN AUSTRALIA (AU)) 10 August 2000 (2000-08-10)

1. The present application does not meet the criteria of **Article 33(1) PCT**, because the subject-matter of **claims 1, 11, 12, 14, 21 - 27, 30, 37-40** is not new in the sense of

without the exercise of inventive skill, in order to solve the problem posed. Subsequently, examples of prior art are provided covering the technical features of most of the dependent claims:

2.2 It is known to the skilled practitioner that dideoxynucleotides lead to the termination of the elongation of a growing nucleotide strand. The addition of a certain ddNTP, for example ddCTP, would therefore terminate more often the strand or template carrying fewer methylated Cytosins, since these Cytosins would be transformed into Uracils/Thymidins with respect to the un-, or hypo-methylated template. In addition the general addition of "terminating dideoxynucleotides" would not contribute to the method of claim 1, aiming at the "detection of cytosine methylation in DNA samples" and therefore **Claim 9** is not inventive with respect to **Article 33(3) PCT**.

2.3 It is known from the prior art, that by using the bisulfite-method to perform methylation analysis, the strand that is less methylated shows more transformation of Cytosins into Uracils and therefore Thymidins than the DNA with a higher degree of methylation. This on the other hand leads to a favouring of the A-T basepairing and due to that, the skilled in the art is aware that the consequence would be a lowering of the melting point of said double stranded DNA. The analysis of the melting curve of double stranded DNA is used to analyze the methylation pattern of said DNA as described in the prior art, c.f. **D5**, the whole document. **Claim 10** therefore is not inventive in the sense of **Article 33(3) PCT** since it is described in the prior art.

2.4 Embedding the DNA in agarose after bisulfite treatment to keep the reaction product concentrated is also a feature known from the prior art, c.f. prior art **D4**, the whole document. **Claim 13** therefore is not inventive in the sense of **Article 33(3) PCT**.

2.5 The use of polymerases devoid of a 5'-3' exonuclease activity is known from the prior art, c.f. document **D10**, where the Stoffel fragment of the Taq DNA polymerase is used to exert sequence-dependent amplification (PCR) inhibition. Since this technique is described in **D10**, **claims 19** and **20** are not inventive in the sense of **Article 33(3) PCT**.

2.6 **Claims 23 - 27** relate to the use of real-time PCR and fluorescence resonance energy transfer (FRET) techniques in the elucidation of the methylation status of DNA in combination with methylation specific PCR methodology (claim 1). The use of these techniques in association with MS-PCR is also known from the prior art, c.f. documents **D11** and **D12**. **Claims 23** and **27** are not inventive in the sense of **Article 33(3) PCT**.

2.7 **Claims 31 - 36** deal with the identification of the amplified products resulting from the methylation specific amplification by means of mass spectrometry with or without mass labels, with primers either being or being not bound to a solid phase. This is described in the prior art, c.f. prior art **D6 - D8**, care for Disclosure of the invention. **Claims 31 - 36** are not inventive in the sense of **Article 33(3) PCT**.

Re Item VIII
Certain observations on the international application

3. **CLARITY** (Article 6 PCT)

3.1 **Claims 1 - 8, 15 - 18, 28 and 29** lack clarity and support with respect to **Article 6 PCT**.

3.2 The wording of **claim 1** is not clear: the aim of claim 1 is to lead "to a preferred amplification of the target DNA over the background DNA" with the use of "at least 2 primer oligonucleotides as well as a polymerase and a nucleotide mixture". The applicant thereby formulates the claim as an effect to be achieved, since it becomes not clear from the wording of said claim what the essential technical features are, that lead to such a result. Claim 1 needs amendment with respect to **Article 6 PCT**. Furthermore the terms "target DNA" and "background DNA" are not defined, said terms need amendment, **Article 6 PCT**. Finally, claim 1 does not clearly specify whether the wording "the composition of which" refers to oligonucleotides, polymerase and nucleotide mixture or to the nucleotide mixture alone, **Article 6 PCT**.

3.3 It is furthermore unclear how the method can work with claim 1 in combination with **claim 2**, when dCTP is completely absent from the reaction pool, thereby taking into consideration that the DNA examined does or does not carry methylated cytosins in a varying amount and is usually never completely methylated on 100% of all Cytosins, **Article 6 PCT**.

3.4 The applicant is to define the relative terms within the claims, such as "comparatively small concentrations" in **claims 3** and **6** for example, **Article 6 PCT**.

3.5 It is furthermore questionable that "a preference for the unmethylated DNA is thus achieved in the present invention by the fact that essentially less dCTP and/or dGTP than ATP and dTTP is added to the PCR reaction". (1) Claim 1 in combination with claims 2 and

3 is unclear: to detect cytosine methylation in DNA samples, the situation after the bisulfite treatment is that most of the cytosines have disappeared due to them being unmethylated and transformed into Uracils. The limiting factor, if at all, would be the concentration of the complementary Adenin base, since if the cytidine base is transformed into a Uracil, being recognized as a Thymidine with respect to the choice of the complementary base by the DNA polymerase, then indeed less Cytidines and more Adenines are needed. (2) if there is a smaller amount of one of the four nucleotides in the pool, then the reaction of both "target" and "background" amplification would stop if the limiting nucleotide was used up, no matter of which origin said "target" or "background" amplification product was. (3) The only difference applicable for a preferential synthesis of either the "target" or "background" amplification product would be a reduced affinity (= Km-value) of the polymerase for one of the four nucleotides. Then, indeed, the incorporation efficiency would be significantly reduced for one of the two synthesized populations and a preference for either product would occur. This though has not been shown by the applicant: the applicant has not shown within the description that the method described in the underlying application (a) indeed works (examples provided are of hypothetical character) and (b) has advantages over the ones of the state of the art, and therefore, in addition to a problem of clarity, there seems to be problem of lack of disclosure with regard to **claims** 1 - 8, **Article** 5 and **6 PCT**.

3.6 **Claims 15 - 18** are unclear and need amendment with respect to the dinucleotide primers and the way they are supposed to work, in addition they are not supported by the description, **Article 6 PCT**.